plus two additional forms representing exons 2 to 4 and
. RNase protection studies have shown further that,
oval, single exon deleted structures are the most
mative isoforms. Moreover, upon their transfection
i of the alternative isoforms were shown to be reactive
AM-1 antibodies, thus confirming the relationship
on of alternatively spliced RNA species and tissue
nice. To examine the function of the alternative
ligands for the well recognized ICAM-1 receptor, LFA-1,
ere produced as ICAM-1-human IgG fusion proteins. Each
ted for its ability to engage LFA-1 upon the surface of
3 lymphoma cells. The results indicate that, in
ular ICAM-1, all alternative isoforms bind to LFA-1 with
ly reduced affinity. This finding, taken together
e distribution of the alternative ICAM-1 isoforms in
its that they are physiological adhesion structures which
ctioning of the immune system of intact animals.

IATION OF FUNCTIONALLY ACTIVE ANTIGEN LLS FROM PERIPHERAL BLOOD CD34⁺ HEMA- IGENITOR CELLS IN CANCER PATIENTS.

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od CD34⁺ progenitor cells (PBPCs) mediate constitution in cancer patients after autologous nd can be expanded ex vivo in the presence of g factors (CSFs). We observed the concomitant xpansion of large numbers of functionally active ng cells (APCs) from these peripheral blood Ne cultured CD34⁺ cells ex vivo in medium r (1) SCF, IL-1ß, IL-3, IL-6, and EPO, as ously to expand clonogenic PBPC [Blood . or (2) SCF, granulocyte-macrophage CSF (GM- necrosis factor-α (TNF-α), or (3) SCF, GM-CSF, Under the culture conditions (1) and (2), the of expanded APCs resembled macrophages ⁺) while under the culture conditions (3) the Cs were typical dendritic cells (DCs) with up to)1a⁺, CD14⁻, HLA-DR^{high}, CD33⁺ non-adherent as demonstrated by electron microscopy, these haracteristic ultrastructural features of DCs. of DCs were observed following 12 to 14 days sion. The cells generated ex vivo from all types powerfull APCs of tetanus-toxoid (TT) and *uberculosis* (PPD) antigens that require antigen resentation. This antigen presenting capacity ens was maintained for at least 38 days of ex ir data demonstrate that DCs can be expanded lood CD34⁺ cells of cancer patients and suggest night be able to present tumor antigens to the Thus, CSF-expanded CD34⁺ autologous PBPCs for vaccination against various malignancies.

similar to both the Tel-PDGFRß and BCR-Abl fusions, but its role in leukemogenesis remains to be characterized. In contrast, a different mechanism of transformation is predicted by ribonuclease protection assays performed in a patient with acute lymphoblastic leukemia. In this patient, we have demonstrated aberrant expression of a fusion transcript encoding not the Tel HLH domain, but rather its DNA binding domain. This fusion resembles previously reported chimeric proteins involving ets DNA-binding domains, such as the EWS-Fli-1 fusion in Ewing's sarcoma. Finally, several patients have been identified in which one *tel* allele is disrupted by a chromosomal translocation, and the other allele is deleted. Although the functional consequences of these translocations have not been fully elucidated, it is possible that loss of Tel function may contribute to the pathogenesis of some leukemias. Chromosome band 12p13 abnormalities, translocations and deletions, are common in lymphoid and myeloid leukemias. The *tel* gene is involved in the majority of these rearrangements, and may be leukemogenic by more than one mechanism.

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AML1B BUT NOT THE AML1/ETO FUSION PROTEIN CAN TRANSACTIVATE THE GM-CSF PROMOTER.

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The t(8;21) translocation, commonly seen in AML of the M2 FAB subtype, generates an AML1/ETO (MTG8) fusion protein containing N-terminal AML1 amino acids and C-terminal ETO amino acids. The wild type AML1 protein is a putative transcription factor, based on structural features, and it is thought that the AML1/ETO fusion protein may interfere with the normal transcriptional activities of AML1. The human AML1 gene encodes several related proteins that specifically bind to the sequence TGT/cGGT. To examine the abilities of the full length AML1B protein (which contains a.a. 1-479), a shorter AML1A isoform (which contains a.a. 1-250), and the AML1/ETO fusion protein (which contains AML1 a.a. 1-177) to stimulate transcription from a hematopoietic growth factor promoter, we performed co-transfection experiments in MLA 144 cells using a human GM-CSF promoter-CAT reporter gene plasmid and expression vectors that contain the cDNAs for one of the above proteins. Our data demonstrate that full length AML1B, but not AML1A or AML1/ETO, transactivates the GM-CSF promoter in a sequence dependent manner, utilizing the TGTGGT sequences contained between base pairs - 68 and - 53. Electrophoretic mobility shift and supershift assays demonstrate the specific binding of AML1 proteins to the GM-CSF promoter TGTGGT sequence, which does not require GM-CSF sequences immediately upstream of this binding site. Competition co-transfection experiments are being performed to determine the effects of AML1/ETO on AML1B mediated transcriptional activation. Our data show that the AML1 protein is a transcriptional activator of the human GM-CSF promoter; the C-terminal part of the protein appears to be required for transcriptional activation.

alternatively spliced form of AML-1 from a human B-cell line. The full length cDNA encodes a protein of 479 amino acids with a predicted molecular mass of 53Kd. This protein, which we have termed AML-1B, is highly homologous to the murine polyoma enhancer binding protein, PEPB2αB and recognizes an AML-1 consensus site. Reconstitution experiments demonstrated that AML-1 and AML-1B produced *in vitro* interacted with CBFβ and co-migrated with endogenous AML-1-containing complexes. Cell localization experiments demonstrate that both AML-1/ETO and AML-1B partition to the nucleus, however AML-1/ETO can be removed from the particulate/nuclear fraction with an ionic wash, whereas AML-1B cannot. Analysis of the transcriptional activity of AML-1 and AML-1B demonstrates that only the larger form, AML-1B, is capable of stimulating transcription from the T-cell Receptor β (TCRβ) enhancer. AML-1/ETO, fails to activate transcription from the TCRβ, although it binds to the AML-1 recognition sequence.

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ONCOGENIC POTENTIALS OF THE AML1/EVI-1 FUSION PROTEIN DERIVED FROM THE t(3;21)(q26;q22) TRANSLOCATION IN BLASTIC CRISIS OF CHRONIC MYELOCYTIC LEUKEMIA.

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We have demonstrated that the t(3;21)(q26;q22), which is usually found in blastic crisis of chronic myelocytic leukemia or myelodysplastic syndrome-derived leukemia, generates an *AML1/EVI-1* chimeric gene and that the t(3;21)-carrying leukemic cell line, named SKH1, expresses the AML1/EVI-1 fusion protein of 180 kD containing amino-terminal half of AML1 including a runt homology domain which is fused to the entire of zinc finger EVI-1 protein (K. Mitani, et al. EMBO J 13; 504, 1994). Thus AML1/EVI-1 fusion protein is a chimeric transcription factor including a runt homology domain from AML1 and two zinc finger domains from EVI-1, totally three DNA binding domains, and an acidic domain from EVI-1 as a transcriptional activation domain. To evaluate the effects of the AML1/EVI-1 fusion protein on cell growth of SKH1 cells, we prepared the synthetic antisense oligonucleotides with 16 nucleotides spanning the junction point between AML1 and EVI-1 sequences and those with 4 point mutations in their sequences as a negative control. The antisense oligonucleotides suppressed ^{3}H-thymidine incorporation in SKH1 cells and decreased the cell number of the cells in comparison with those including 4 point mutations, suggesting that the AML1/EVI-1 fusion protein should play a crucial role in the growth of leukemic cells with the t(3;21) translocation. To demonstrate the transforming activity of the fusion protein, AML1/EVI-1 was introduced retrovirally into Rat1 cells. Cells expressing the fusion product formed colonies in soft agar, indicating the oncogenic potentials of the AML1/EVI-1 fusion protein. Moreover, the introduction of AML1/EVI-1 into Rat1 clones harboring BCR/ABL conferred enhanced ability for anchorage independent growth. The analysis using deletion mutants showed that the second zinc finger domain within the EVI-1 was the functional region critical fot transformation. All these data suggest that the AML1/EVI-1 could play an important role in leukemic progression of chronic myelocytic leukemia or myelodysplatic syndrome, depending on its DNA-bindind domain of EVI-1.